REGULAR ARTICLE

blood advances

T-replete HLA-matched grafts vs T-depleted HLA-mismatched grafts in inborn errors of immunity

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Key Points

- $CD3^{+}TCR\alpha\beta$/CD19–depleted mismatched grafts result in comparable survival to unmanipulated HLA-matched grafts in young children with IEI.
- Graft-versus-host disease rate is comparable between T-replete and T-depleted graft recipients, with more viremia observed in T-depleted graft recipients.

Hematopoietic cell transplantation (HCT) has become standard-of-care for an increasing number of inborn errors of immunity (IEI). This report is the first to compare transplant outcomes according to T-cell–replete (ie, T-replete) HLA-matched grafts using alemtuzumab (n = 117) and T-cell–depleted (ie, T-depleted) HLA-mismatched grafts using T-cell receptor-αβ (TCRαβ)/CD19 depletion (n = 47) in children with IEI who underwent first HCT between 2014 and 2019. All patients received treosulfan-based conditioning except patients with DNA repair disorders. For T-replete grafts, the stem cell source was marrow in 25 (21%) patients, peripheral blood stem cell (PBSC) in 85 (73%), and cord blood in 7 (6%). TCRαβ/CD19 depletion was performed on PBSCs from 45 haploidentical parental donors and 2 mismatched unrelated donors. The 3-year overall survival (OS) and event-free survival for the entire cohort were 85% (77%-90%) and 79% (69%-86%), respectively. Analysis according to age at transplant revealed a comparable 3-year OS between T-replete grafts (88%; 76%-94%) and T-depleted grafts (87%; 64%-96%) in younger patients (aged <5 years at HCT). For older patients (aged >5 years), the OS was significantly lower in T-depleted grafts (55%; 23%-78%) compared with T-replete grafts (87%; 68%-95%) ($P = .03$). Grade III to IV acute graft-versus-host disease was observed in 8% of T-replete marrow, 7% of T-replete PBSC, 14% of T-replete cord blood, and 2% of T-depleted PBSC ($P = .73$). Higher incidence of viremia ($P < .001$) and delayed CD3 reconstitution ($P = .003$) were observed after T-depleted graft HCT. These data indicate that mismatched donor transplant after TCRαβ/CD19 depletion represents an excellent alternative for younger children with IEI in need of an allograft.

Introduction

Inborn errors of immunity (IEI) encompass a group of >400 inherited disorders.[1] IEI lead to severe problems and may be fatal in severe phenotypes. Although hematopoietic cell transplantation (HCT) has

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The full-text version of this article contains a data supplement.

always been standard-of-care for infants with severe combined immunodeficiency (SCID), it is now an established curative treatment of an increasing number of non-SCID IEI. For many non-SCID IEI such as chronic granulomatous disease, studies have shown that long-term survival, disease outcomes, and quality of life are better in transplanted patients compared with nontransplanted patients who were treated with conventional therapy using antimicrobial prophylaxis.[2] In recent years, HCT has developed from a treatment of last resort into the standard of care that corrects the defects of immunity in children and young adults with non-SCID IEI. Many studies have also shown that younger age at transplant is significantly associated with better survival and outcomes.[3] However, ~25% to 60% of eligible pediatric patients for HCT do not have a suitably well-matched donor.[4] An alternative is to use a mismatched related or unrelated donor and deplete the T lymphocytes in the graft before infusion. Historically, the major challenges of using such mismatched donors for HCT are graft-versus-host disease (GvHD), graft failure, and high transplant-related mortality. In the absence of a suitably matched donor, physicians are reluctant to offer a curative transplant procedure to children with IEI, leading to prolonged periods of illness, poor quality of life, significant psychosocial problems, and reduced life expectancy.

A new graft selection method has now enabled selective depletion of the T-cell receptor-αβ-bearing ($TCR\alpha\beta^+$) cells that cause GvHD and $CD19^+$ (B) cells, decreasing the risk of Epstein-Barr virus (EBV)-driven posttransplant lymphoproliferative disease. In this method, the cellular product contains $CD34^+$ progenitors, $TCR\gamma\delta^+$ T lymphocytes, other progenitor cells, monocytes, and dendritic cells, which might have engraftment facilitating effects, with a low incidence of GvHD in children with malignant and nonmalignant disorders. To address the question of whether transplant outcomes after a TCRαβ/CD19–depleted graft are comparable to a matched family or unrelated donor, we analyzed the outcome of consecutive transplants in children with IEI over the past 6 years in Newcastle upon Tyne, United Kingdom.

Methods

Patients and methods

Between January 2014 and December 2019, a total of 184 children with IEI underwent their first allograft at the Great North Children's Hospital. Sixteen patients who received an unmanipulated T-cell–replete (ie, T-replete) mismatched graft and 4 patients with SCID who received stem cell infusion without conditioning or serotherapy were excluded from this study. A total of 164 patients were included in the final analysis. The clinical and laboratory data were retrieved from the transplantation database, patients' medical files, and laboratory records. Written informed consent was obtained from the parents or legal guardians of the patients as per institutional practice for HCT.

Donor selection, stem cell source, conditioning regimen, and GvHD prophylaxis

Donor selection was based on allele-level high-resolution HLA typing for HLA-A, -B, -C, -DQ, and -DR loci. The donor hierarchy for SCID was: (1) matched family donor (MFD); (2) matched unrelated cord blood (CB); and (3) haploidentical donor. The donor hierarchy for non-SCID IEI was: (1) MFD; (2) matched unrelated donor (MUD); (3) mismatched family/unrelated donor; and (4) haploidentical donor. From 2018 onward, a haploidentical donor was preferred to a mismatched unrelated donor except if parents were not suitable as donors. The donor selection criteria for a haploidentical donor were as follows: (1) noncarrier donor for X-linked diseases; (2) maternal donor for a patient with SCID with maternal fetal engraftment; and (3) donor with a better HLA match.

In our center, peripheral blood stem cell (PBSC) graft has been the first choice of stem cell source with 10/10 HLA-matched donors since 2011 except when using young sibling donors aged <16 years.[5] From 2011 onward, the $CD3^+$ cell dose in T-replete PBSC was capped at 5×10^8/kg, and the maximum dose of $CD34^+$ cells were given within this dose of $CD3^+$ cells. All patients received fludarabine-treosulfan (Flu-Treo)–based chemotherapy except 3 patients with DNA repair disorders who received fludarabine and low-dose cyclophosphamide, and 1 patient with X-linked lymphoproliferative disease who received fludarabine-melphalan. Additional thiotepa was added for patients with diseases that were associated with a high risk of graft failure. In T-replete HLA-matched grafts, alemtuzumab 1 mg/kg was given for all PBSC recipients while alemtuzumab 0.6 mg/kg was given for family donor marrow and unrelated CB recipients. Posttransplant GvHD prophylaxis was cyclosporine (CSA) and mycophenolate mofetil. For T-cell–depleted (ie, T-depleted) mismatched grafts, patients received standardized conditioning, which consisted of fludarabine, treosulfan, ATG (Grafalon [Neovii Biotech], 5 mg/kg per dose on days −4, −3, and −2), and 1 dose of rituximab (200 mg/m^2) for SCID. Thiotepa was added for non-SCID IEI. One T-depleted graft recipient received alemtuzumab (1 mg/kg). The graft manipulation strategy was TCRαβ and CD19 depletion as previously described.[6] Thirteen patients received a CaspaCIDe gene-modified T-lymphocyte add-back as part of a phase 1/2 clinical trial (#NCT03301168). When the TCRαβ/CD19 depletion strategy was first implemented in 2012, all patients received CSA prophylaxis. In patients who received TCRαβ cells $>5 \times 10^4$/kg, both CSA and mycophenolate mofetil were given as GvHD prophylaxis. No GvHD prophylaxis was given since 2015 in patients receiving TCRαβ cells $<5 \times 10^4$/kg.

Supportive care

Surveillance for cytomegalovirus (CMV), adenovirus, EBV, human herpes virus type 6 (HHV6) viremia, respiratory, and gastrointestinal viruses was performed weekly. All patients received antimicrobial prophylaxis against fungi, *Pneumocystis jiroveci* (PCP), and human herpesvirus reactivation. All patients received immunoglobulin replacement until normal immunoglobulin M levels were evident. Donor hematopoietic chimerism was monitored by using molecular techniques.

Definition and end points

The main outcomes of interest were overall survival (OS) and event-free survival (EFS) and cumulative incidence (CIN) of GvHD. OS was defined as survival from first HCT to last follow-up or death. An event was defined as death, graft failure, or second procedures for slipping chimerism. Other end points assessed were as follows: (1) time to neutrophil recovery (first day of achieving a neutrophil count $\geq 0.5 \times 10^9$/L for 3 consecutive days); (2) incidence of transplant-related complications as defined and graded according to existing institutional guidelines at the time of HCT; (3) immune reconstitution; and (4) degree of donor hematopoietic chimerism at the most recent assessment.

For pretransplant patient-related risk factors, the following factors were included: (1) active infection (viremia, *Cryptosporidium*, PCP, disseminated bacillus Calmette-Guérin, mycobacteria, and serious bacterial infection requiring IV antibiotics) within 100 days before or at transplant; (2) chronic diarrhea; (3) autoimmunity/autoinflammatory; (4) lung damage (abnormal lung function test result or bronchiectasis or oxygen dependency at HCT); (5) liver damage (proven fibrosis/sclerosing cholangitis on liver biopsy or deranged liver function test result [bilirubin >1.5 upper limit of normal or alanine aminotransferase/aspartate aminotransferase >2.5 upper limit of normal within 100 days before HCT]); (6) renal impairment within 100 days before HCT; (7) neurologic disorders secondary to infection, bleeding, vasculitis, or encephalitis (disease-related congenital malformation or developmental delay was not included); (8) growth failure; and (9) pretransplant malignancy.

Statistical analysis

Quantitative variables are described with median and range, and categorical variables are reported with counts and percentages. The association between variables was assessed with the use of the Kruskal-Wallis test for continuous variables and the χ^2 test for categorical variables. Subgroup differences in OS and EFS were evaluated by using the log-rank test. Competing risk methods were used for the CIN of acute GvHD (aGvHD) and viremia, with death as a competing event, and graft failure was added as a competing event for CIN of aGvHD. Subgroup differences in aGvHD and viremia were evaluated by using Gray's test. All estimates are reported with 95% confidence intervals (CIs). A matched-pair analysis of CD3 reconstitution was conducted in T-replete HLA-matched PBSC survivors (n = 28) and T-depleted mismatched survivors without T-lymphocyte add-back. The 2-patient series were matched for age at transplant and diagnosis. Multilevel mixed effects modeling was performed for the longitudinal analysis of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, natural killer cells, and HLA-DR. All *P* values quoted are two-sided, with a level of significance of .05. Statistical analyses were performed by using STATA 14.2 (StataCorp).

Results

Patient and transplantation characteristics

Patient and transplantation characteristics are summarized in Table 1 and supplemental Table 1. There were no significant differences in age at diagnosis and age at transplant between T-replete HLA-matched graft recipients and T-depleted HLA-mismatched graft recipients. The median age at transplant for the entire cohort was 2.5 years (range, 0.14-18.1 years). For pretransplant risk factors, there was no significant difference in number of pretransplant risk factors between T-replete HLA-matched graft recipients and T-depleted mismatched graft recipients for both SCID and non-SCID IEI (supplemental Table 2). T-depleted HLA-mismatched graft recipients (29 of 47 [62%]) had a significantly higher rate of active infection within 100 days of HCT compared with T-replete HLA-matched family (13 of 37 [35%]) or unrelated donor (28 of 80 [35%]) recipients (*P* = .008) (supplemental Table 3). There was a greater proportion of patients with chronic diarrhea in T-depleted HLA-mismatched graft (17 of 47 [36%]) and T-replete HLA-matched unrelated graft (31 of 80 [39%]) recipients compared with T-replete HLA-matched family graft (6 of 37 [16%]) recipients (*P* = .04). The proportions of patients with autoimmunity/autoinflammation, lung damage, liver damage, renal impairment, neurologic disorders, growth failure, or pretransplant malignancy were comparable between T-replete HLA-matched graft recipients and T-depleted HLA-mismatched graft recipients. There was no significant difference in the proportion of patients with pretransplant history of viremia (CMV, EBV, adenovirus, and HHV6) between T-replete HLA-matched graft (24 of 117 [21%]) and T-depleted HLA-mismatched graft recipients (13 of 47 [28%]) (*P* = .41) (supplemental Table 4).

For T-replete grafts, the stem cell source was marrow in 25 (21%) patients, PBSC in 84 (72%), and CB in 7 (6%). TCR$\alpha\beta$/CD19 depletion was performed on PBSCs from 45 haploidentical parental donors and 2 mismatched unrelated donors. Of 47 T-depleted graft recipients, 33 (70%) had no post-HCT GvHD prophylaxis, and 14 (%) received GvHD prophylaxis (8 CSA only; 6 CSA/mycophenolate mofetil). For T-replete graft recipients, 93 received Flu-Treo/alemtuzumab, 17 Flu-Treo/thiotepa/alemtuzumab, 3 fludarabine-cyclophosphamide/alemtuzumab (1 DNA ligase IV deficiency; 2 Nijmegen breakage syndrome), 1 fludarabine-melphalan/alemtuzumab, and 3 patients with SCID received alemtuzumab only. For T-depleted graft recipients, 11 with SCID received Flu-Treo/ATG/rituximab and 36 with non-SCID IEI received Flu-Treo/thiotepa/ATG/rituximab, except for 1 patient who received alemtuzumab as serotherapy.

The graft composition is shown in Figure 1. Between T-replete PBSCs and T-depleted PBSCs, total nucleated cell dose was significantly higher in T-replete PBSCs (*P* = .002), but the $CD34^+$ cell dose was comparable between both T-replete PBSCs and T-deplete PBSCs (Figure 1A-B). $CD3^+$ cells (*P* < .001) and $CD19^+$ cells (*P* < .001) were lowest in T-depleted PBSCs (Figure 1C-D).

Engraftment and transplant-related complications

Table 2 summarizes the engraftment kinetics and transplant-related complications. The rates of neutrophil and platelet engraftment were comparable between T-depleted PBSCs and T-replete PBSCs and significantly earlier compared with T-replete marrow and T-replete CB (Figure 1E-F).

The CIN of grade II to IV aGvHD was 18% (95% CI, 7-48) in T-replete marrow, 26% (95% CI, 16-41) in T-replete PSBCs, 14% (95% CI, 2-79) in T-replete CB, and 18% (95% CI, 9-39) in T-depleted PBSCs (*P* = .73) (Figure 2A). Grade III to IV aGvHD was observed in 8% (95% CI, 2-30) of T-replete marrow, 7% (95% CI, 3-17) of T-replete PBSCs, 14% (95% CI, 2-79) of T-replete CB, and 2% (95% CI, 3-17) of T-depleted PBSCs (*P* = .73) (Figure 2B). Only one T-depleted graft recipient who did not receive ATG due to concerns about drug-resistant CMV disease developed grade III to IV aGvHD. None had chronic GvHD in the entire cohort.

With routine surveillance for CMV, adenovirus, EBV, and HHV6, the CIN of any reactivation of viremia at 6 months after transplant was significantly higher in T-depleted grafts (80%; 70%-99%) compared with T-replete grafts (55%; 46%-64%) (*P* < .001) (Figure 3). Compared with T-replete graft recipients, a greater proportion of T-depleted graft recipients had adenoviremia (*P* = .004) and HHV6 viremia (*P* = .002) (Table 2). All CB recipients were patients with SCID, and only 1 had new-onset viremia after transplant.

Table 1. Patient and transplantation characteristics according to donor type (n = 164)

Characteristic	All	T-replete HLA-matched graft		T-depleted HLA-mismatched graft	*P*
		MFD	MUD		
No.	164	37	80	47	
Male sex, n (%)	97 (59)	16 (43)	52 (65)	29 (62)	NS
Diagnosis, n (%)					NS
SCID	35 (21)	10 (27)	14 (18)	11 (23)	
Non-SCID IEI	129 (79)	27 (73)	66 (83)	36 (77)	
Age at diagnosis, median (range), y					
SCID	0.2 (at birth-1.0)	0.13 (at birth-0.7)	0.3 (at birth-0.8)	0.3 (at birth-1.04)	NS
Non-SCID IEI	1.9 (at birth-17.1)	2.1 (at birth-12.8)	1.5 (at birth-14.3)	2.0 (0.1-17.1)	NS
Age at transplant, median (range), y					
SCID	0.4 (0.14-1.4)	0.3 (0.2-0.8)	0.54 (0.14-1.4)	0.65 (0.14-1.4)	NS
Non-SCID IEI	4.1 (0.2-18.1)	4.32 (0.2-16.8)	4.7 (0.3-17.2)	3.1 (0.2-18.1)	NS
Interval between diagnosis and transplant, median (range), y					
SCID	0.2 (0.02-1.2)	0.16 (0.11-0.32)	0.24 (0.02-1.2)	0.22 (0.13-0.8)	NS
Non-SCID IEI	1.2 (0.1-17.2)	0.8 (0.2-12.8)	1.3 (0.1-17.2)	1.2 (0.1-4.6)	NS
No. of pretransplant risk factors, median (range)					
SCID	2 (0-4)	1 (0-3)	2 (0-4)	2 (0-3)	NS
Non-SCID IEI	2 (0-6)	1 (0-5)	2 (0-6)	2 (0-5)	NS
Graft composition					
Median TNC (range), $\times 10^8$/kg	12.0 (1.0-96.0)	7.6 (1.4-22.8)	13.9 (1.2 – 37.9)	10 (1.0-96.0)	<.0001
Median CD34$^+$ (range), $\times 10^6$/kg	12.4 (0.5-60.9)	6.7 (1.7-25.7)	13.5 (0.5-8.7)	18.4 (0.8-60.9)	<.0001
CD3$^+$ (range), $\times 10^8$/kg	1.9 (0.02-14.4)	0.9 (0.2-7.7)	4.6 (0.3-8.7)	0.2 (0.02-14.4)*	<.0001
CD19$^+$ (range), $\times 10^7$/kg	5.25 (0.0008-27.0)	5.0 (1.1-21.0)	9.4 (0.7-27.0)	0.007 (0.008-0.11)	<.0001

NS, not significant; TNC, total nucleated cell.
*Median TCR$\alpha\beta^+$ cell dose, 4.1 $\times$ 10^4/kg (range, 0.06-20 $\times$ 10^4/kg).

A greater proportion of T-replete marrow (n = 16 [64%]) and T-replete PBSC (n = 57 [67%]) recipients developed acute kidney injury (serum creatinine levels increased >2 times from baseline creatinine before transplant) during transplant, compared with T-replete CB recipients (n = 2 [28%]) and T-depleted graft recipients (n = 11 [23%]) (*P* = .001). Of 86 patients with acute kidney injury, 7 (8% [4 T-replete PBSCs, 1 T-replete CB, and 2 T-depleted PBSCs]) required renal replacement therapy. There was no significant difference in occurrence of transplant-associated microangiopathy between T-replete graft recipients (marrow, 0; PBSC, n = 4 [5%]; CB, n = 1 [14%]) and T-depleted graft recipients (n = 4 [9%]) (*P* = .33). Only 1 patient developed macrophage activation syndrome after T-replete PBSCs for juvenile idiopathic arthritis.

Transplant survival

The 3-year OS and EFS for the entire cohort were 85% (77%-90%) and 79% (69%-86%), respectively. Analysis according to age at transplant revealed a comparable 3-year OS between T-replete grafts (88%; 76%-94%) and T-depleted grafts (87%; 64%-96%) in patients who were aged <5 years at transplant. For older patients (aged >5 years), the OS was significantly lower in T-depleted grafts (55%; 23%-78%), compared with T-replete grafts (87%; 68%-95%) (*P* = .03) (Figure 2C). A similar pattern was observed in EFS (Figure 2D). Among patients who received a T-replete HLA-matched donor transplant, OS and EFS were comparable between MFD (n = 37) and MUD (n = 80). OS was 91% (74%-97%) for MFD and 89% (80%-95%) for MUD (*P* = .76), whereas EFS was 78% (47%-92%) for MFD and 85% (73%-92%) for MUD (*P* = .89).

Regarding pretransplant risk factors, there were no significant differences between T-replete HLA-matched graft recipients and T-depleted HLA-mismatched graft recipients in patients who were aged >5 years at HCT (supplemental Tables 5 and 6), and between patients aged <5 years and patients aged >5 years after T-depleted HLA-mismatched graft HCT. The exception was that a greater proportion of T-depleted recipients aged >5 years had autoimmunity (5 of 13 [38%] vs 1 of 34 [3%] in younger T-depleted recipients; *P* = .001) before transplant (supplemental Tables 7 and 8). In patients who were aged >5 years and received T-depleted grafts, a greater proportion of deceased recipients (4 of 5 [80%]) had >2 risk factors compared with the survivors (4 of 8 [50%]), although the number was too small to reach statistical significance (supplemental Table 9).

A trend toward lower survival was observed in T-depleted graft recipients with viremia (75%; 54%-88%) compared with T-depleted graft recipients without viremia (88%; 41%-98%), and in T-replete graft recipients with viremia (89%; 75%-95%) and without viremia (86%; 72%-94%) (*P* = .38). All deceased T-depleted recipients died of infection and its associated complications (supplemental Table 10).

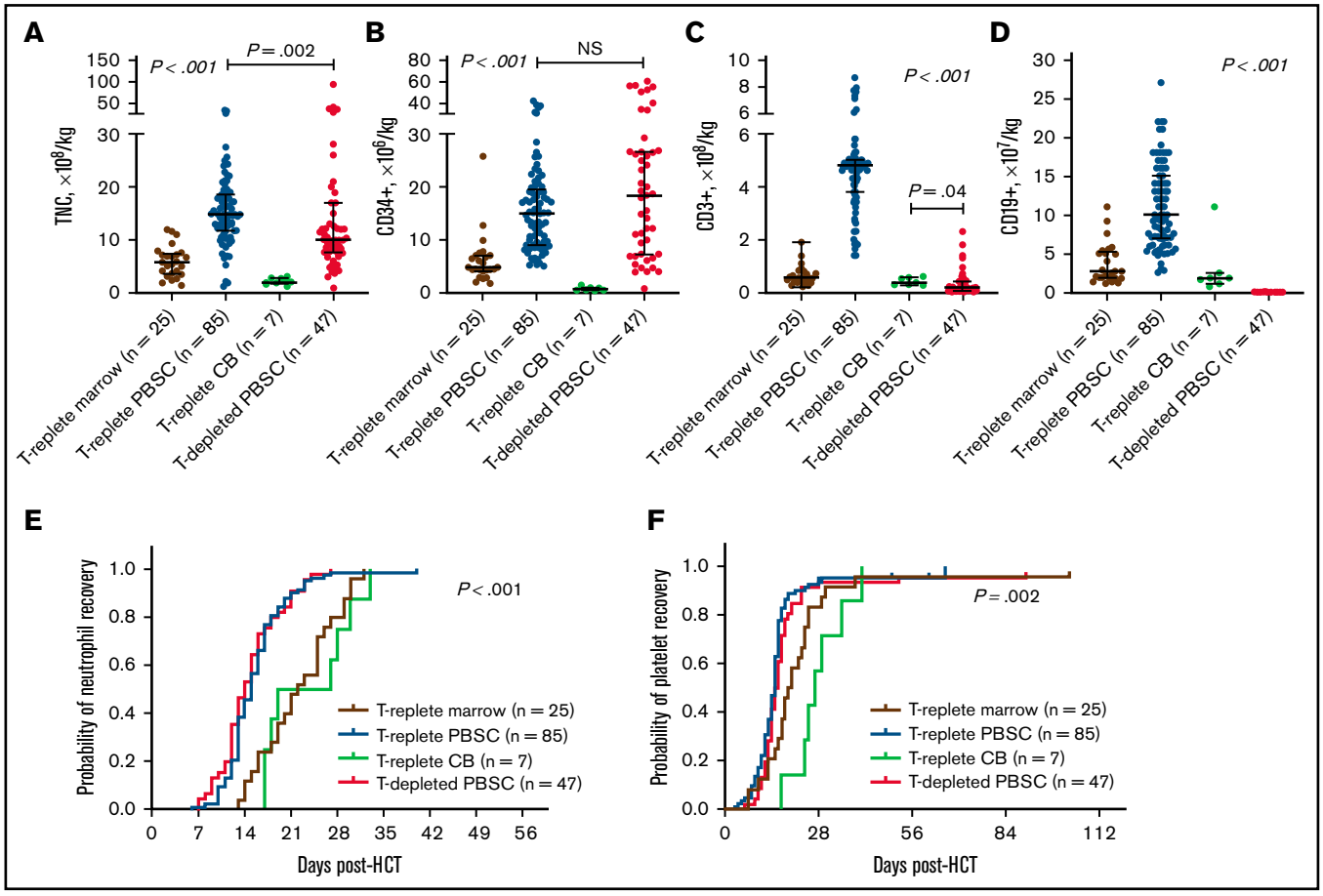

Figure 1. Graft composition and engraftment kinetics according to T-replete marrow, T-replete PBSC, T-replete CB, and T-depleted PBSC. (A) Total nucleated cell (TNC) dose, $\times 10^8$/kg. (B) CD34$^+$ cells, $\times 10^6$/kg. (C) CD3$^+$ cells, $\times 10^8$/kg. (D) CD19$^+$ cells, $\times 10^7$/kg. (E) Neutrophil recovery. (F) Platelet recovery. NS, not significant.

Six (5%) grafts failed in the entire cohort, 4 (5%; all secondary autologous reconstitution) after T-replete PBSCs and 2 (4%; 1 secondary aplasia; 1 secondary reconstitution) after T-depleted PBSCs. All received a second HCT: 5 received a TCRαβ/CD19–depleted parental graft, and one received T-replete PBSCs from an HLA-MUD. All survived except 1 patient, who died of cerebral hemorrhage before engraftment.

Hospital stay and parenteral nutritional support

The median duration of hospitalization was 46 days (range, 13-187 days) for T-replete MFD, 60 days (range, 14-365 days) for T-replete MUD, and 62 days (range, 27-365 days) for T-depleted mismatched donor recipients (P = .15) (supplemental Figure 1A). The proportion of patients requiring intensive care was 11% (4 of 37) for T-replete MFD, 11% (9 of 80) for T-replete MUD, and 17% (8 of 47) for T-depleted mismatched donor (P = .62) recipients. The proportion of patients requiring parenteral nutrition support was significantly higher in T-depleted mismatched donor transplants (77% [36 of 47]) compared with T-replete MFD (41% [15 of 37]) and T-replete MUD (51% [41 of 80]) (P = .003). There was no significant difference in duration of parenteral nutrition between T-depleted mismatched donor (median, 53 days; range, 17-196 days), T-replete MFD (median, 41 days; range, 9-172 days), and T-replete MUD (median, 60 days; range, 11-189 days) (P = .52) (supplemental Figure 1B).

Immune reconstitution and donor chimerism

To compare the immune reconstitution, a matched-pair analysis was performed in survivors of T-depleted grafts without T-lymphocyte add-back (n = 28 received ATG) and T-depleted PBSCs (n = 28 received alemtuzumab 1 mg/kg). The median day to CD3 >200 cells/μL was 66 (range, 33-133 days) in T-replete graft patients and 89 days (range, 34-397 days) in T-depleted graft patients (P = .003) (supplemental Figure 2). Immune-reconstitution kinetics within the first 6 months is illustrated in Figure 4. T-depleted graft recipients had significantly lower CD3$^+$ (Figure 4A), CD4$^+$ (Figure 4B), CD8$^+$ (Figure 4C), and activated (Figure 4D) T-lymphocyte counts at months 2 and 3 posttransplant. There was no significant difference in natural killer cell reconstitution (Figure 4E) between T-replete and T-depleted graft recipients. CD19$^+$ B lymphocytes were significantly lower in T-depleted graft recipients at month 2 posttransplant, but B-lymphocyte reconstitution was comparable between T-replete and T-depleted graft recipients after 3 months' post-HCT (Figure 4F). TCRαβ and TCRγδ reconstitution for T-depleted survivors without T-lymphocyte add-back is shown in

Table 2. Engraftment kinetics and transplant-related complications according to T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts

Transplant outcomes	T-replete HLA-matched graft			T-depleted HLA-mismatched graft (n = 47)	*P*
	Marrow (n = 25)	PBSC (n = 85)	CB (n = 7)		
Engraftment kinetics					
Days to neutrophil recovery, median (range)	22 (13-32)	15 (6-40)	27 (17-33)	14 (0-27)	<.001
Days to platelet recovery, median (range)	19.5 (7-103)	15 (3-66)	27 (17-41)	15 (6-90)	.002
aGvHD, % (95% CI)					
1-year CIN grade II-IV aGvHD	18 (7-48)	26 (16-41)	14 (2-79)	18 (9-39)	NS
1-year CIN grade III-IV aGvHD	8 (2-3)	7 (3-17)	14 (2-79)	2 (3-17)	NS
Organ involved in aGvHD (all stages), n (%)					
Skin	6 (24)	28 (33)	2 (29)	17 (36)	NS
Gut	0	2 (2)	1 (14)	1 (2)	NS
Liver	0	2 (2)	0	0	–
Chronic GvHD, n (%)	0	0	0	0	–
New onset of viremia after HCT, n (%)					
CMV viremia	6 (24)	17 (20)	0	14 (30)	NS
Adenoviremia	5 (20)	19 (22)	0	22 (47)	.004
HHV6 viremia	4 (16)	24 (28)	0	24 (51)	.002
EBV viremia	5 (20)	11 (13)	1 (14)	8 (17)	NS
TMA, n (%)	0	4 (5)	1 (14)	4 (9)	.33
Acute kidney injury, n (%)	16 (64)	57 (67)	2 (28)	11 (23)	<.001
Graft failure, n (%)	0	4 (5)	0	2 (4)	NS
No. of deaths, n (%)	1 (4)	9 (10)	1 (14)	8 (17)	NS
Cause of death					
GvHD	0	1	0	0	
Multiorgan failure	1	2	0	3	
Infection	0	3	0	3	
Others	0	3*	1†	2‡	

NS, not significant; TMA, transplant-associated microangiopathy.
*Neuromyelitis optica spectrum disorder, n = 1; encephalopathy, n = 1; posttransplant lymphoproliferative disease, n = 1.
†Pulmonary TMA, n = 1.
‡Encephalopathy, n = 1; cerebral hemorrhage, n = 1.

supplemental Figure 3. The TCRαβ proportion increased gradually and exceeded TCRγδ proportion after 3 months' posttransplant.

The median follow-up of surviving patients (n = 146) was 2.8 years (range, 0.5-6.5 years). Data on latest donor chimerism after first HCT were available in 141 patients. A greater proportion of T-depleted graft recipients had full donor myeloid chimerism >95% (30 of 39 [77%]) compared with T-replete graft recipients (62 of 102 [61%]) (P = .04) (Figure 5A; supplemental Figure 4A). For T-lymphocyte chimerism, 90% (35 of 39) of T-depleted graft recipients had full donor T-lymphocyte chimerism >99% compared with 55% (56 of 102) in T-replete graft recipients (Figure 5B; supplemental Figure 4B).

Discussion

This study reports the first series comparing transplant outcomes between T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts in children with IEI who underwent transplant between 2014 and 2019. Treosulfan-based conditioning was used for both T-replete and T-depleted grafts. Thiotepa was given for all non-SCID IEI in T-depleted grafts but in a subset of non-SCID IEI with a high risk of graft rejection in T-replete grafts. The survival after T-depleted grafts was comparable to that in T-replete grafts in younger children with IEI but inferior in older children with IEI. A greater proportion of older T-depleted graft recipients had autoimmunity and received immunosuppressive therapy before transplant compared with younger T-depleted graft recipients. We observed a significantly higher incidence of adenoviremia and HHV6 viremia after T-depleted grafts but a similar incidence of CMV viremia compared with that of T-replete graft recipients. Delayed CD3 reconstitution remains a significant issue after T-depleted grafts. We also found that T-replete PBSCs using alemtuzumab were not significantly associated with increased aGvHD, compared with T-replete marrow and CB; this is consistent with the report of Shaw et al[7] in patients with leukemia. In our cohort, no recipients of T-replete PBSCs had chronic GvHD.

HCT has become a curative option for a variety of otherwise incurable diseases such as high-risk leukemia and many nonmalignant

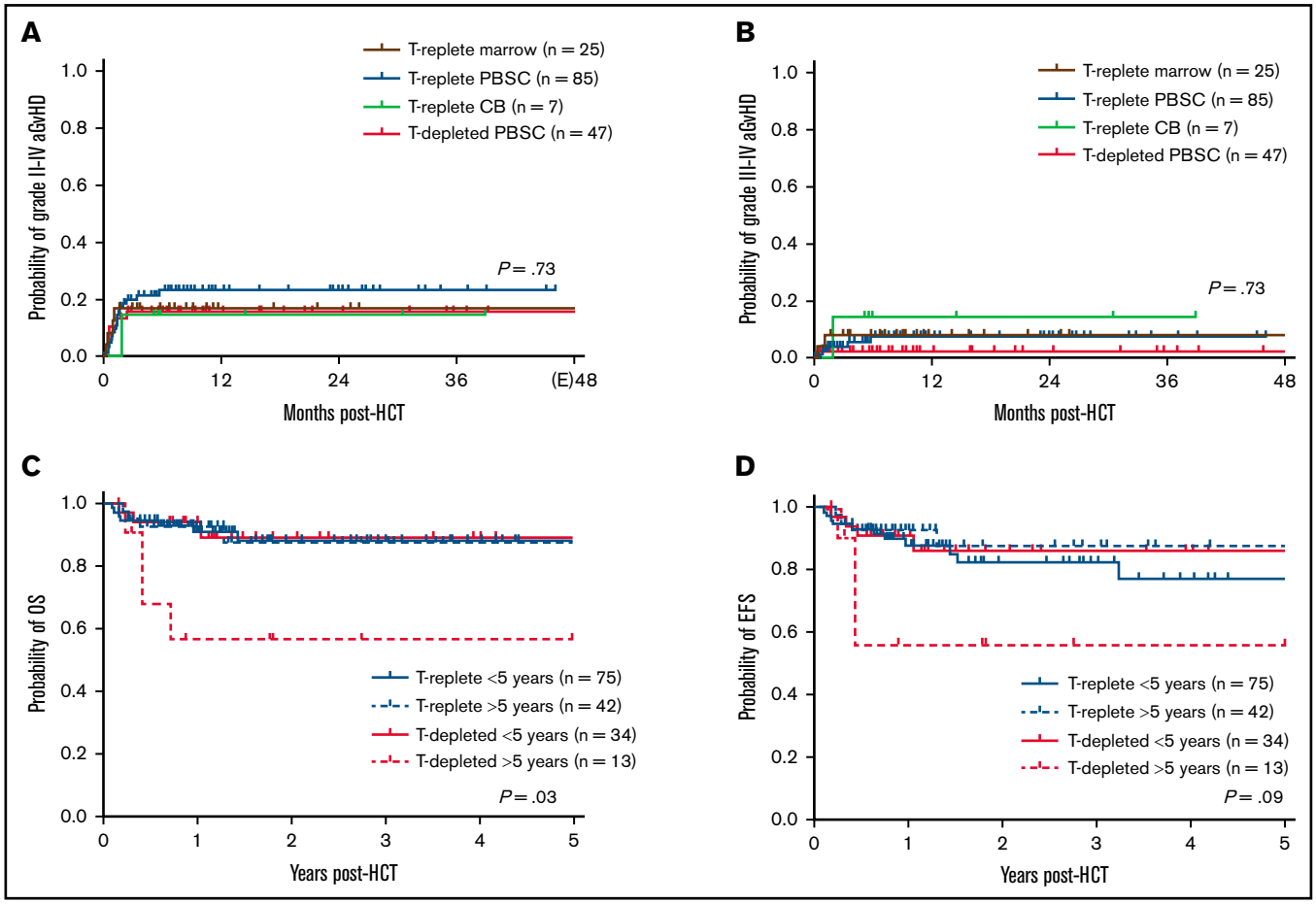

Figure 2. Cumulative incidence of aGvHD and survival according to T-replete and T-depleted grafts. (A) Grade II to IV aGvHD. (B) Grade III to IV aGvHD. (C) OS according to age at transplant. (D) EFS according to age at transplant.

conditions. It has been increasingly used as standard-of-care for an increasing number of IEI. Unfortunately, only ~1 in 4 patients who need an HCT will have a HLA-matched sibling available to become a stem cell donor.[8] The likelihood of finding an optimal unrelated donor varies among racial and ethnic groups; for example, as high as 75% in White subjects of European descent to as low as 16% among ethnic groups of South or Central American descent.[4] Transplantation from a haploidentical donor has some unrivaled advantages, including highly motivated and readily available donors; in addition, in the setting of malignancy, a haploidentical donor allows the selection of a donor with the highest degree of mismatch in their natural killer cell immunoglobulin-like receipt repertoire, which provides a better graft-versus-tumor effect.[9] Historically, the major challenges of using such mismatched haploidentical donors for HCT are GvHD, graft failure, and high transplant-related mortality. In the absence of a suitably matched donor, physicians are reluctant to offer a curative transplant procedure to children with IEI, leading to prolonged periods of illness, poor quality of life, significant psychosocial problems, and reduced life expectancy. Locatelli et al[10] reported comparable risks of nonrelapse mortality and relapse in children with acute leukemia between $CD3^+$ TCRαβ/CD19–depleted haploidentical donor graft and matched family and unrelated donor recipients. In this report, we found comparable outcomes between T-depleted HLA-mismatched grafts and T-replete HLA-matched grafts in younger children with IEI.

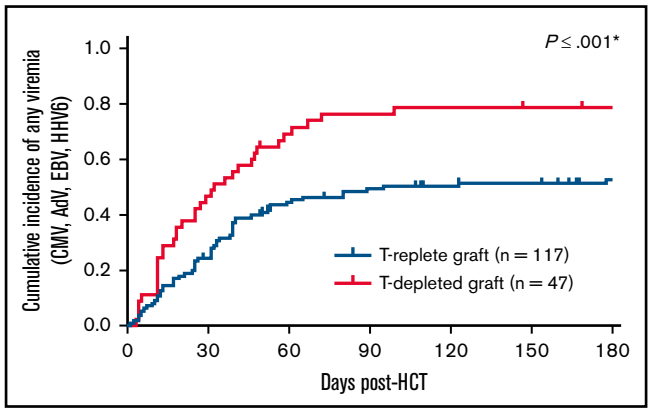

Figure 3. Cumulative incidence of viremia (CMV, adenovirus [AdV], EBV and HHV6) according to T-replete and T-depleted grafts.

The current strategy using TCRαβ/CD19 depletion has a comparable incidence of GvHD to T-replete HLA-matched grafts, but the immune reconstitution and generation of a broad TCR repertoire, which are required for normal immunity, are delayed.[11] This

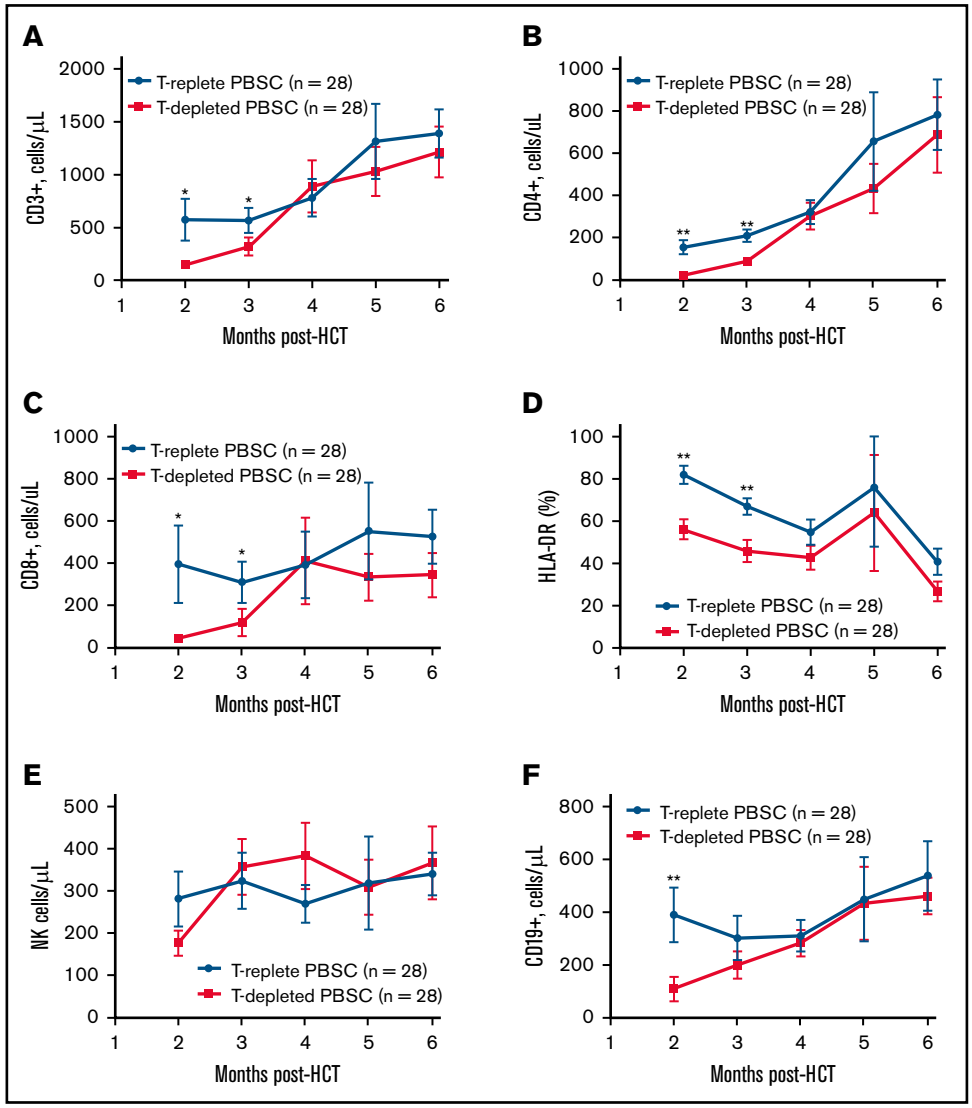

Figure 4. A matched-pair analysis for immune reconstitution kinetics posttransplant. Means (± standard error of the mean) $CD3^+$ (A), $CD4^+$ (B), $CD8^+$ (C), activated T lymphocytes (D), natural killer (NK) cells (E), and $CD19^+$ lymphocytes (F) measured at different time points posttransplant. $^*P < .05$; $^{**}P < .001$.

is reflected in the significant rate of viral infections in our cohort, especially adenoviremia and HHV6 viremia. Of note, the incidence of acute kidney injury was significantly lower in T-depleted graft patients, likely due to the fact that the majority of these patients did not receive any calcineurin inhibitors for GvHD prophylaxis. In long-term survivors, the donor myeloid and T-lymphocyte chimerism were better in the T-depleted graft group. One possible explanation for this finding is that additional thiotepa was given to patients with non-SCID IEI for T-depleted grafts to prevent graft rejection, which is more myeloablative compared with Flu-Treo–based conditioning. Although Leiper et al[12] showed that treosulfan-based conditioning conferred a more favorable outcome for gonadal reserve, the long-term impact of additional thiotepa on fertility function is unknown.

Although the introduction of $CD3^+$ TCRαβ/CD19 depletion has transformed our practice for children with IEI, improved strategies are required to accelerate immune recovery and reduce viral infection. A number of such emerging strategies include the generation of donor-derived cytotoxic T lymphocytes against multiple viruses, rivogenlecleucel (genetically modified αβ T-lymphocyte receptor bearing cells with an added caspase suicide gene), and photodynamic purging.[13,14] These approaches might be promising, but they are complex and time-consuming, and additional harvests from donors are required. Depletion of naive ($TCR\alpha\beta^+CD45RA^+$) T lymphocytes has been developed as a new method of graft selection with the potential to confer improved reactivity to pathogens by memory T lymphocytes ($TCR\alpha\beta^+CD45RO^+$), without conferring an increased risk of GvHD. Recent trials in children with malignancy show positive benefits from $CD45RO^+$ memory T-lymphocyte add-back with improvement in immune reconstitution, reduction in incidence and severity of viral infection, and lower transplant-related mortality.[15-17] To date, there is no prospective trial in children with IEI using TCRαβ/CD19–depleted mismatched graft with $CD45RO^+$ memory T-lymphocyte add-back.

In conclusion, our data indicate that through a more advanced strategy of graft selection, suitable donors, whether mismatched related

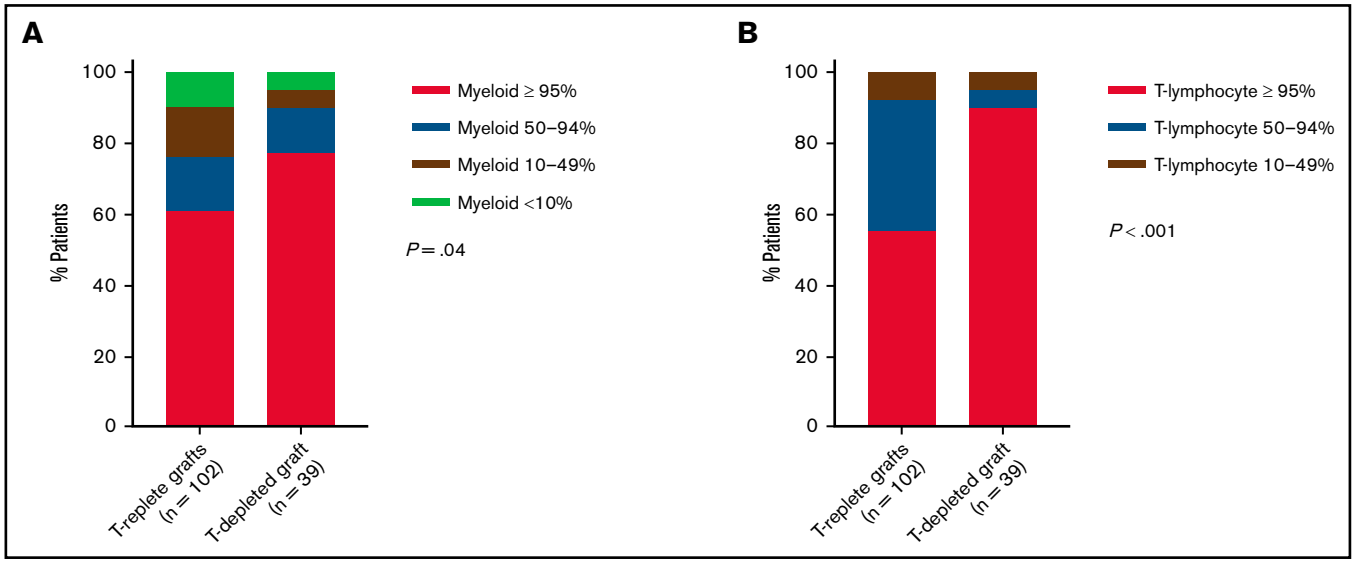

Figure 5. The latest donor chimerism in T-replete and T-depleted grafts. (A) Myeloid donor chimerism. (B) T-lymphocyte chimerism.

or unrelated can always be found, offering the opportunity to transplant almost every children with IEI in need of an allograft, with an expected outcome comparable to that obtained with an HLA-matched donor in younger children. We are now setting up a new prospective trial, based on $CD45RO^{+}$ memory T-lymphocyte add-back, with the aim of accelerating recovery of adaptive immunity and reducing the incidence of viral infection in patients who receive in vitro T-depleted grafts. Demonstration of efficacy and safety would allow physicians to refer IEI patients earlier to transplant, avoiding many IEI-related complications as well as transplant-related morbidity and mortality that rise with each year of delay. For infants who are diagnosed with IEI through newborn and family screening, transplant can be performed as soon as possible before any infection or organ damage. Every child with IEI deserves a cure with transplant: the major obstacle of "no suitable donor" will be eliminated if this clinical trial shows promising outcomes.

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Authorship

Contribution: S.H.L. developed the conceptualization of the research, collected the data, performed the statistical analysis, interpreted the data, and prepared the manuscript; M.S. contributed to the conceptualization of the research, manuscript writing, and critical review at every stage of the research; S.G., I.P.-H., D.D., R.P.P., H.W., K.C., R.J., S.W., and S.B.-F. collected the data and reviewed the manuscript; and A.R.G., T.F., Z.N., S.H., A.C., T.R.L., S.O., E.W., and M.A. critically reviewed the manuscript.

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